



UNITED STATES AIR FORCE RESEARCH LABORATORY

DERMAL ABSORPTION OF COMP B AND CRB-12 IN ISOLATED RAT SKIN

J. McDougal
D. Pollard
D. Dodd

MANTECH-GEO-CENTERS JOINT VENTURE
P.O. BOX 31009
DAYTON, OH 45437-000*

R. Davis

OPERATIONAL TOXICOLOGY BRANCH, AFRL/HES
2856 G STREET
WRIGHT PATTERSON AFB, OH 45433-7400

September 2000

Interim Report - October 1999 - August 2000

20060630288

Human Effectiveness Directorate
Deployment and Sustainment Division
Operational Toxicology Branch
2856 G Street
Wright-Patterson AFB OH 45433-7400

Approved for public release; distribution is unlimited.

STINFO COPY

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Air Force Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Service
8725 John J. Kingman Rd., Ste 0944
Ft. Belvoir, Virginia 22060-6218

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Air Force Research Laboratory.

TECHNICAL REVIEW AND APPROVAL

AFRL-HE-WP-TR-2001-0058

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR



RICHARD R. STOTTS, Ph.D.
Branch Chief, Operational Toxicology Branch
Air Force Research Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 2000	3. REPORT TYPE AND DATES COVERED Interim Report - October 1999 - August 2000
4. TITLE AND SUBTITLE Dermal Absorption of Comp B and CRB-12 in Isolated Rat Skin			5. FUNDING NUMBERS Contract F41624-96-9010 PE 62202F PR 1710 TA 1710D WU 1710D413
6. AUTHOR(S) McDougal, J., Pollard, D., Davis, R., Dodd, D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Human Effectiveness Directorate Air Force Research Laboratory Wright-Patterson AFB, OH 45433-7400			8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) ManTech Geo-Centers Joint Venture PO Box 31009 Dayton, OH 45437-0009			10. SPONSORING/MONITORING AGENCY REPORT NUMBER AFRL-HE-WP-TR-2001-0058
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.			12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 words) Potential health hazards of new Army weapon systems are of concern to health and safety professionals, the general public, and weapon system developers. One concern is the potential for dermal absorption from materials and chemicals. High explosives, such as CBR-12, which is intended for use in the 60mm M720E1 and M768 mortar shells, may be modified so that they will be less likely to explode from the unplanned stimuli. The purpose of this effort was to estimate the impact of such a modification on dermal absorption. We investigated the penetration of powdered explosives through dermatomed rat skin in static diffusion cells for up to six hours. We compared "Composition B" with a replacement explosive, CBR-12. We detected penetration of very small amounts of one of the components of each explosive. Steady state flux of trinitrotoluene from "Composition B" was 1.14 ug/cm ² /hr and steady state flux of dinitroanisole from CBR-12 was 0.74 ug/cm ² /hr. These rates of penetration are not expected to be hazardous.			
14. SUBJECT TERMS Composition B CBR-12			15. NUMBER OF PAGES 18
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL

THIS PAGE INTENTIONALLY LEFT BLANK.

TABLE OF CONTENTS

SECTION	PAGE
TABLE OF CONTENTS.....	iii
LIST OF TABLES.....	iv
PREFACE.....	v
INTRODUCTION.....	1
Explosives.....	1
Skin as a Barrier.....	3
Factors Affecting Dermal Absorption.....	4
METHODS.....	5
Skin Preparation.....	5
Static Diffusion Cells.....	6
Analytical Methods.....	7
RESULTS AND DISCUSSION.....	8
Composition B replacement (CBR-12).....	8
Composition B.....	9
Pure Dinitroanisole.....	10
SUMMARY.....	11
REFERENCES.....	13

LIST OF TABLES

TABLE	TITLE	PAGE
Table 1	Comparison of percent composition of each of the major components of mortar explosives tested.....	2

PREFACE

This technical report addresses the absorption of a proposed military mortar explosive through skin. This research was designed to provide quantitative information that is useful for assessing the potential hazards of exposures to proposed and existing mortar explosives in the manufacturing process. Assessment of the potential for dermal absorption of chemicals that may come in contact with the skin is essential for assuring the safety of production workers during the manufacturing and loading process.

This research was accomplished at the Operational Toxicology Branch, Human Effectiveness Directorate of the Air Force Research Laboratory. This research was completed under Man Tech – Geo-Centers Joint Venture Contract (F41624-96-C-9010). Lt. Col. (sel) Stephen L. Channel served as Contract Technical Monitor for the U.S. Air Force, Air Force Research Laboratory. The Product Manager for Mortar Systems at Picatinny Arsenal (AMSTA-AR-WEP) provided the funding for this project.

The animal use described in these studies was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

INTRODUCTION

Humans may be exposed to explosives in the manufacture, loading, assembly, transportation and use of mortar shells. Skin contact with the explosives themselves and their residue on mortars and other surfaces are a potential route of absorption for the chemical components of explosives. The Army has an initiative to improve explosives so that they are less likely to inadvertently detonate. In many cases, this means that the explosives will be reformulated. Therefore, it is important to determine the level of dermal absorption hazard for new explosives so that the appropriate protective equipment can be determined. It is also important that a new explosive be compared with the existing one to assure that hazards are not increased. The purpose of this project is to provide weapons developers for the Product Manager for Mortar Systems at Picatinny Arsenal (AMSTA-DSA-MO) the toxicity information that will allow the determination of the safe use of "Composition B Replacement" (CBR-12) in mortar shells.

Explosives

CBR-12 is the replacement for the current explosive (composition B) used in 60mm high explosive mortar rounds. Table 1 shows the differences in chemical composition of these explosives. Both explosives contain RDX, as the largest component by weight. The replacement, CBR-12 doesn't have TNT but does have dinitroanisole and ammonium perchlorate, which are not in the current explosive. The other components are minor and are not of concern.

Table 1 Comparison of percent composition of each of the major components of mortar explosives tested.

Component	Composition B	CBR-12
RDX (1,3,5-trinitro-1,3,5-triazacyclohexane)	~60%	~36%
TNT (2,4,6-trinitrotoluene)	~39%	0%
2,4-Dinitroanisole	0%	~34%
Ammonium perchlorate	0%	~30%
Wax, desensitizing	≤ 1%	0%
MNA (N-methyl-4-nitroaniline)	0%	≤ 1%

When the exposure route is the skin, there is very little systemic toxicity information about RDX available. There are some older human reports that are available. In one study, a man was exposed to RDX via a skin patch and there was no irritation two days later (von Oettingen et al., 1949). In another report, workers reported dermatitis and conjunctivitis after exposures in RDX plants (Army 1974). Acute, intermediate and chronic animal studies have been completed for the Army (Army 1974). The intravenous LD₅₀ for RDX in dimethyl sulfoxide was 18.7 mg/kg in mice and 25.1 mg/kg in guinea pigs. When applied dermally, RDX (in acetone, cyclohexanone or dimethyl sulfoxide) did not cause physiological responses in dogs or changes in blood cell counts or blood enzymes in rabbits. The authors interpreted the lack of response as lack of systemic absorption. This study was inconclusive because it was difficult to determine whether RDX alone was responsible for the toxicity.

TNT, on the other hand, has lots of information about dermal toxicity and dermal absorption in humans. Occupational exposures to TNT can result in

hematopoietic effects and hepatitis (ATSDR, 1995) but it is often hard to separate the dermal and inhalation effects because of the volatility of TNT.

Dinitroanisole and ammonium perchlorate have no toxicity information available from the dermal route of exposure.

Of the components shown in Table 1, the American Congress of Governmental Industrial Hygienists (ACGIH, 2000) has determined a TLV™ for TNT (0.1 mg/m³) and RDX (0.5 mg/m³). Both TNT and RDX have skin notations, which means that there is a "potential significant contribution to the overall exposure by the dermal route". The other components do not have workplace standards.

Skin as a Barrier

The skin is a good barrier that inhibits the penetration of most liquids and particles into the body, but it is the largest organ in the body and its surface area provides ample potential for exposure to the environment. When skin is not damaged or broken most chemicals are prohibited from entering the body by the outermost layer of the skin, the stratum corneum. The stratum corneum is a densely packed layer of dead keratinized cells that are held together in a lipid "cement". Chemicals can only penetrate this barrier by passive diffusion. If chemicals get through the stratum corneum, they can be picked up by the capillaries in the underlying dermis and enter the blood stream where they may cause systemic toxicity.

Dermal absorption of chemicals in the solid form is not well understood, primarily because of the poor quality of studies in the literature. Most studies apply the solid chemical in a vehicle, such as acetone and express the results as percentage of applied dose absorbed. Vehicles, acetone in particular,

enhance the rate of absorption of chemicals across the skin because of the effect of the vehicle, itself, on the skin. Studies that express the results as "percent absorbed" are only useful if they mimic the human exposure of interest. Flux across the skin is the most useful way to express penetration data from solid chemicals. :

Factors Affecting Dermal Absorption

Chemicals diffuse through the skin at different rates based on their molecular weight, lipid solubility and ionization (Dugard and Scott, 1984). Chemicals that have an ionic charge do not penetrate the skin to any appreciable extent. Chemicals that have a low molecular weight and therefore a small molecular volume diffuse through the stratum corneum better than chemicals that have a large molecular weight. Chemicals that are lipid soluble penetrate the stratum corneum better than chemicals that are water soluble. Water soluble chemicals are excluded because the most important function of the skin is to keep bodily fluids in and bath water out.

Fick's law is often used to mathematically describe the absorption of chemicals across the skin (Flynn et al., 1974). Fick's law states that the flux of a chemical across a membrane is proportional to the concentration difference across a membrane (C), the affinity of the chemical for the membrane (K_m), and the molecular characteristics of the chemical (D). Flux is also indirectly proportional to the thickness of the membrane (l).

$$Flux = \frac{DK_m}{l}(C_{out} - C_{in}) \quad (1)$$

When we are dealing with a mixture such as these solid explosives, the flux from the mixture could be more or less than the flux from the pure chemical depending on the other components in the mixture, which may act as a vehicle

in addition to diluting the chemical. Changing the concentration of a propellant component from 5% to 10% would theoretically double the flux rate according to equation 1 above. This is an important concept that can be used to compare changes in propellant formulations.

METHODS

Skin Preparation

Male rats (CDF® F-344/CrlBr, Charles River Breeding Laboratories), weighing 270-366g, were sacrificed using CO₂. The back of the animal was closely clipped of fur with Oster® animal clippers (McMinnville TN) and a #40 blade, taking care not to damage the skin. An Oster® finishing clipper (0.22mm) was used to carefully remove the fur stubble. A thin cardboard circle, the size of the diameter of the outside edge of the diffusion cell was used as a template to mark a circle on the midscapular area of the rat's back with a waterproof marker. The marked skin containing the future exposure site was gently excised from the back using scissors and blunt dissection. The skin was placed stratum corneum side up on a 5 x 30 cm oak board and dermatomed to 560 micrometers using a Padgett dermatome (Kansas City MO). The skin was trimmed with scissors to match the size of the circular mark and placed on the glass receptor chamber that was previously filled with receptor solution. To reduce regional variability each diffusion cell contained the skin from one rat.

Static Diffusion Cells

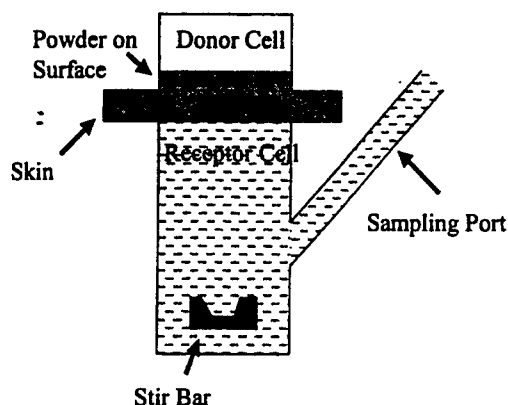


Figure 1. Schematic of Static Diffusion Cell.

Static Diffusion cells with 4.9 cm² skin exposure area (Figure 1) were used to determine flux of each component of the explosives. These brown glass cells (Crown Glass Company, Somerville NJ) fit 9 to a countertop console which provides magnetic stirring of the receptor solution and fluid flow to the water jackets (not shown in Figure 1) around the receptor cells. These diffusion cells have a 14.1 mL stirred receptor compartment right under the skin with a 7 cm long sampling port. The receptor compartment was filled with a solution of 6% Volpo 20 (polyethylene glycol-20 oleyl ether, Croda, Mill Hill PA) in physiological saline. Skin temperature in the cells was controlled at 32°C with a Haake DC3 circulating water bath (Karlsruhe, Germany). The donor chamber was placed on top of the skin and secured by screw clamps. Half a gram of powdered explosive was placed in the donor chamber. The receptor solution was sampled at hour intervals for 6 hours. The experiment was repeated on 2 different days and the results were pooled. Steady-state flux was determined from the slope of the mass absorbed (per area exposed) over time.

Analytical Methods

Receptor solution and standard samples for RDX, TNT and dinitroanisole were run on a Hewlett-Packard (Palo Alto, Ca) Series 1050 High Performance Liquid Chromatograph (HPLC). A Brownlee, 220 X 4.6 mm Spheri 5, RP- 18 reverse phase column was used for separation. Column temperature was 32C and the carrier was 70% methanol and 30% water with a flow of 0.4 ml/min. The detector was a variable wavelength, ultra-violet/visible detector. Injection size was one μ L. The detector wavelength used for RDX and TNT was 233 nm. 215 nm was the wavelength used for dinitroanisole. Approximate retention times were 10 min for RDX, 14 min for dinitroanisole and 15 min for TNT. No commercial source was found for RDX, but the mortar mixtures were received with reported analyses. The standards were prepared in volpo saline from the mortar material using the stated concentrations. Standards and samples were run in the same manner with no treatment and no dilution. Detection limits were 0.15 μ g/mL, 53.9 ng/mL, and 0.23 μ g/mL for RDX, TNT and dinitroanisole, respectively.

Receptor solution and standards for ammonium perchlorate were performed with a Dionex DX-300 (Dionex Corporation, Sunnyvale, CA) liquid chromatographic system equipped with a conductivity detector. The chromatographic system consisted of an advanced gradient pump (Dionex, AGP standard size), conductivity detector (CDM-3), anion self regenerating suppressor, ASRS (4-mm), for the reduction of the background conductivity of the eluent, autosampler (AS-3500), computer interface ACI and software Autolon 450. Separation was performed on a Dionex AS11 analytical column (4x250 mm) preceded by a Dionex AG11 guard column (4x50 mm). Ten μ L samples were injected. The mobile phase consisted of 100mM sodium

hydroxide in water. The flow rate was set at 1 mL/minute. Sodium perchlorate standards were prepared in deionized water. Volpo saline receptor samples were not treated nor diluted. They were injected into the HPLC system in a manner identical to that of the standards. The detection limit for perchlorate was 200 ng/mL.

RESULTS AND DISCUSSION

Composition B replacement (CBR-12)

When CBR-12 was placed on the skin in the static diffusion cells, the only component of the explosive that could be found in the receptor solution was dinitroanisole. Neither RDX or ammonium perchlorate could be detected. Figure 2 shows the time course of dinitroanisole penetration through the skin in the diffusion cell. Dinitroanisole concentrations in the receptor solution were not significantly different from baseline until two hours into the exposure. The average total mass absorbed over a 6 hour period in 16 diffusion cells was $18.8 \pm 5.5 \mu\text{g}$. Steady-state flux of dinitroanisole over the period from two to six hours (where absorption was linear) was $0.74 \mu\text{g}/\text{cm}^2/\text{hr}$.

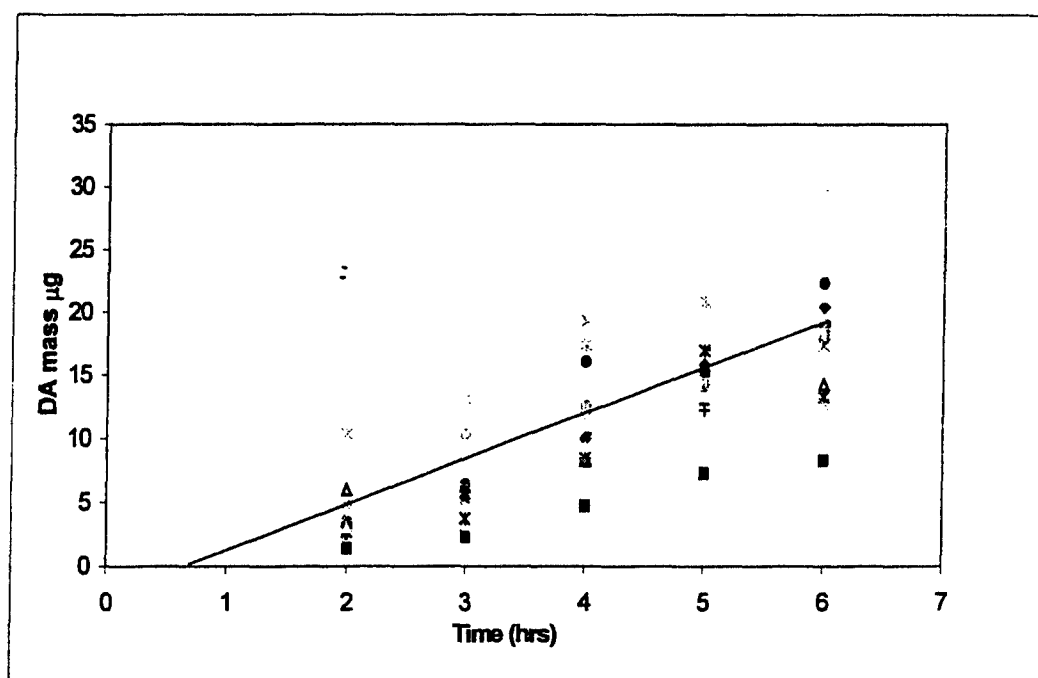


Figure 2. Mass of dinitroanisole (DA) in the receptor solution of diffusion cells with CBR-12 in the donor cell. Each individual cell is shown as a symbol ($n=16$) and the line is the best fit to all the points ($R^2=0.994$).

Figure 2 suggests that the variability seen is due to the skin thickness or treatment rather than the analytical methods, because there are some cells which are consistently high and other cells which are consistently low. The percentage of RDX, dinitroanisole and ammonium perchlorate in CBR-12 is nearly equal (Table 1) but only dinitroanisole was found in the receptor solution. If the other components of CBR-12 (RDX and ammonium perchlorate) penetrated the skin but remained below the level of detection, their fluxes would have to be less than $0.086 \mu\text{g}/\text{cm}^2/\text{hr}$ and $0.114 \mu\text{g}/\text{cm}^2/\text{hr}$, respectively.

Composition B

When composition B was placed on the skin in the static diffusion cells, the only component of the explosive that could be found in the receptor solution was trinitrotoluene. The only other major component, RDX, could not be

detected. Figure 3 shows the time course of trinitrotoluene mass penetrated through the skin in the diffusion cell. Trinitrotoluene concentrations were not significantly different than baseline until two hours into the exposure. The average total mass absorbed in 16 diffusion cells was $28.2 \pm 6.7 \mu\text{g}$ in six hours. Flux of trinitrotoluene over the period from two to six hours was $1.14 \mu\text{g}/\text{cm}^2/\text{hr}$.

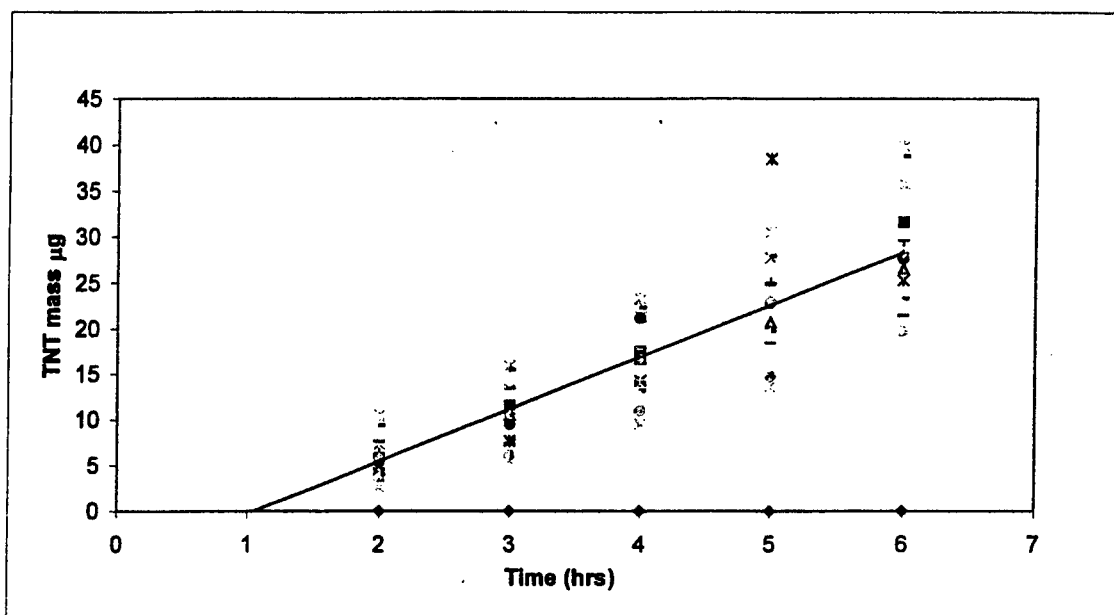


Figure 3. Mass of trinitrotoluene (TNT) in the receptor solution of diffusion cells with composition B in the donor cell. Each individual cell is shown as a symbol ($n=16$) and the line is the best fit to all the points ($R^2=.998$)

The percentage of RDX in Composition B is about 50% greater than the percentage of TNT in Composition B (Table 1), yet no RDX was detectable in the receptor solution. This indicates that TNT penetrates the skin much better than RDX. If RDX penetrated the skin but remained below the level of detection, the steady state flux would be less than $0.086 \mu\text{g}/\text{cm}^2/\text{hr}$.

Pure Dinitroanisole

When pure dinitroanisole powder was placed on the skin in the static diffusion cells, the rate of penetration was determined for comparison with CBR-12. Figure 4 shows the mass of dinitroanisole penetrated through the skin in the diffusion cell. The average total mass absorbed in each of 16 diffusion cells was $36.8 \pm 8.0 \mu\text{g}$ in six hours. Flux of dinitroanisole over the period from two to six hours was $1.55 \mu\text{g}/\text{cm}^2/\text{hr}$.

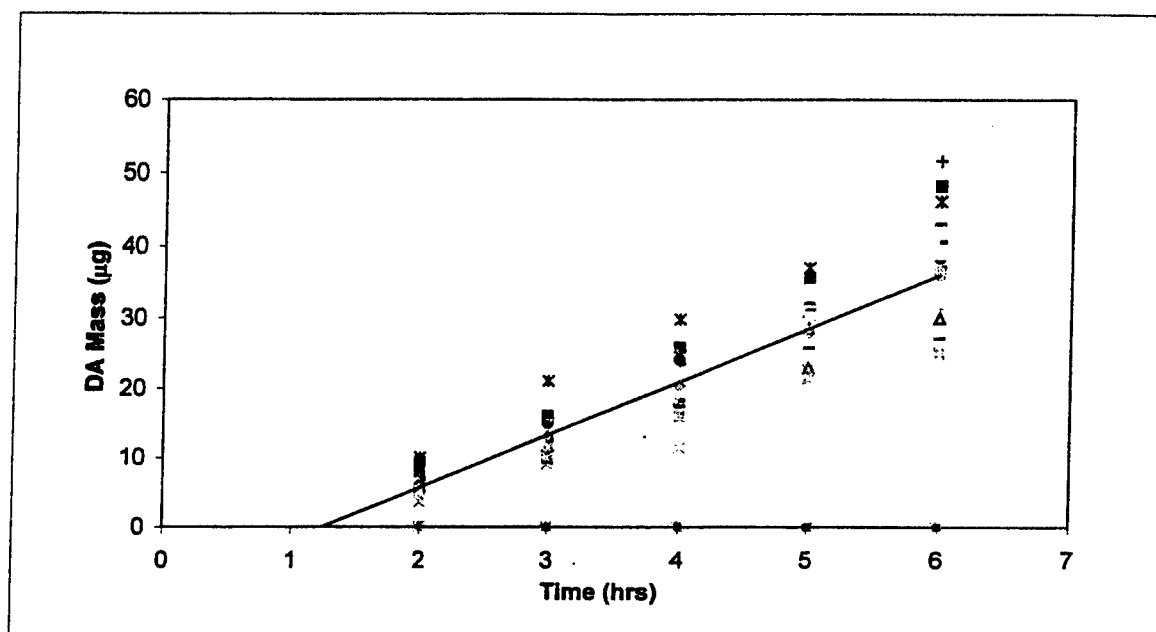


Figure 4. Mass of dinitroanisole (DA) in the receptor solution of diffusion cells with pure dinitroanisole in the donor cell. Each individual cell is shown as a symbol ($n=16$) and the line is the best fit to all the points ($R^2=0.998$).

The flux of dinitroanisole from pure dinitroanisole was approximately twice the flux of dinitroanisole from CBR-12 (1.55 vs $0.74 \mu\text{g}/\text{cm}^2/\text{hr}$). The difference in percentage of dinitroanisole in CBR-12 versus pure chemical was about 3-fold (34% vs 100%), so the result is not entirely explained by mass difference.

SUMMARY

Static diffusion cell studies with dermatomed rat skin show that very small amounts of components of the current mortar explosive and the proposed mortar explosive may penetrate the skin. Trinitrotoluene, which makes up 39% of Composition B, penetrates the skin at a steady state flux of $1.14 \mu\text{g}/\text{cm}^2/\text{hr}$. Dinitroanisole, which makes up 34% of the proposed replacement (CBR-12), has a steady state flux of $0.74 \mu\text{g}/\text{cm}^2/\text{hr}$. The flux of these explosive components is about the same order of magnitude as the flux of hydrocarbon components of jet fuel (0.3 to $1.65 \mu\text{g}/\text{cm}^2/\text{hr}$) investigated in the same system (McDougal et al., 2000).

REFERENCES

- ACGIH Threshold Limit Values for Chemical Substances and Physical Agents - Biological Exposure Indices, American Conference of Governmental Industrial Hygienists. (2000).
- Army. The toxicology of Cyclotrimethylenetrinitramine (RDX) and Cyclotetramethylenetetranitramine (HMX) solutions in Dimethylsulfoxide (DMSO), cyclohexanone, and acetone. Edgewood Arsenal AD-780 010, April 1974.
- ATSDR Toxicological Profile for 2,4,6-trinitrotoluene. US Department of Health and Human Services, June 1995.
- Dugard, P.H. and Scott, R.C.. Absorption through the skin. In Chemotherapy of Psoriasis, H.P. Baden (ed), Pergamon Press, Oxford. pp. 125-144 (1984).
- Flynn, G.L., S.H. Yalkowsky and T.J. Roseman. Mass transport phenomena and models: theoretical concepts. *J. Pharm. Sci.* **63**:479-509 (1974).
- McDougal, J.N., D.L. Pollard, W.H. Weisman, C.M. Garrett, and T.E. Miller. Assessment of Skin Absorption and Penetration of JP-8 Jet Fuel and its Components. *Toxicological Sciences* **55**:247-255 (2000).
- Von Oettingen, WF, DD Donahue, H Yagoda et al.. Toxicity and potential dangers of cyclotrimethylenetrinitramine (RDX). *J. of Industrial Hygiene and Toxicology* **31**:21-31 (1949).